



# Plasma microfibrillar-associated protein 4 is not prognostic of emphysema progression but is associated with cardiovascular disease history and mortality in COPD patients

To the Editor:

Chronic obstructive pulmonary disease (COPD) is characterised by chronic airflow limitation and inflammation of the airways and lung parenchyma. COPD is associated with many comorbidities, especially cardiovascular disease (CVD), which share similar risk factors with COPD [1].

Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix (ECM) protein with the capacity to bind to various ECM fibres [2, 3] and to activate integrin receptors [4, 5]. MFAP4 has been associated with protection of skin elasticity [3] and protection from airway enlargement *in vivo* [6]. We have previously demonstrated that circulating MFAP4 may serve as a biomarker for liver fibrosis [7–9], but it may also serve as a biomarker for COPD [10] and for mortality in CVD [11].

The aim of this study was to evaluate further the relationship between circulating MFAP4 and lung function decline, and to investigate the potential association with emphysema progression, CVD history, Agatston score (as a surrogate of coronary atherosclerosis) and mortality in a cohort of patients with COPD from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study.

Heparin-anticoagulated plasma (hp)MFAP4 concentration was determined in a subset of stable COPD patients from the ECLIPSE cohort consisting of 996 COPD patients. Patient characteristics, circulating inflammatory markers, computed tomography (CT) and coronary artery calcium score have been presented previously [12]. hpMFAP4 was detected by an AlphaLISA (PerkinElmer, Skovlunde, Denmark) immunoassay as described previously [13]. Quality controls were included in each plate and consisted of one human serum pool prepared to contain a low content of MFAP4 (Q<sub>low</sub>) and two pools spiked with purified rMFAP4 (Q<sub>mid</sub> and Q<sub>high</sub>). The three quality controls were included in each plate. The interassay coefficient of variation (CV) calculated from 15 consecutive runs was 11.2% for Q<sub>high</sub>, 10.2% for Q<sub>mid</sub> and 8.9% for Q<sub>low</sub>. Intra-assay CV calculated from 32 runs was 5.3%.

Fisher's exact test was used to test differences in frequency of current smokers, history of CVD and death between hpMFAP4 quartiles in the complete cohort. Regression coefficients and 95% confidence intervals were generated by multiple linear regressions with the various outcomes in table 1 as independent variables. The COPD population was stratified into current smokers and former smokers, and the analyses were performed with hpMFAP4, sex, age and body mass index (BMI) as independent variables. Odds ratio were generated by logistic regression for the categorical variables CVD history and death, and using the same independent variables. The Benjamini–Hochberg procedure was applied with a false discovery rate of 0.10 to prevent  $\alpha$ -error accumulation due to multiple testing.



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**Circulating MFAP4 is a relevant biomarker to identify COPD patients at risk of death and cardiovascular comorbidity after smoking cessation** <http://ow.ly/6vnL30o8t1g>

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TABLE 1 Associations between chronic obstructive pulmonary disease outcomes and heparin-anticoagulated plasma microfibrillar-associated protein 4 in patients stratified into current smokers and former smokers

Subjects	All <sup>#</sup>		Former smokers <sup>¶</sup>		Current smokers <sup>+</sup>		
	B-coefficient (95% CI)	p-value	B-coefficient (95% CI)	p-value	B-coefficient (95% CI)	p-value	
<b>Year 0 (enrolment)</b>							
6MWD m	979	1.16 [0.05–2.27]	0.04	1.30 [0.01–2.60]	0.049	0.66 [–1.60–2.90]	NS
mMRC	961	–0.004 [–0.01–0.01]	NS	0.0006 [–0.01–0.01]	NS	–0.02 [–0.04––0.002]	0.03
SGRQ	965	–0.20 [–0.37––0.02]	0.03	–0.10 [–0.30–0.10]	NS	–0.52 [–0.88––0.15]	0.006
BODE index	939	–0.02 [–0.04––0.001]	0.04	–0.02 [–0.05––0.0001]	0.049	–0.01 [–0.05–0.03]	NS
GOLD stage	996	–0.01 [–0.02––0.006]	<0.001	–0.02 [–0.02––0.01]	<0.001	–0.003 [–0.02–0.009]	NS
PD-FEV1 mL	996	6.9 [2.5–11.3]	0.002	9.2 [4.1–14.3]	<0.001	–1.3 [–10.2–7.6]	NS
PD-FEV1 % predicted	996	0.25 [0.10–0.39]	0.001	0.31 [0.15–0.48]	<0.001	0.02 [–0.27–0.30]	NS
LAA%	887	–0.12 [–0.22––0.01]	0.03	–0.15 [–0.28––0.02]	0.02	0.02 [–0.17–0.21]	NS
CVD history	996	1.04 <sup>§</sup> [1.02–1.07]	<0.001	1.06 <sup>§</sup> [1.03–1.08]	<0.001	1.01 <sup>§</sup> [0.96–1.05]	NS
Agaston score total	362	7.3 [–5.3–19.9]	NS	2.9 [–11.3–17.2]	NS	22.5 [–3.7–48.6]	NS
<b>Year 1 (MFAP4 measurement)</b>							
IL-6 pg·mL <sup>–1</sup>	860	0.01 [–0.11–0.13]	NS	–0.02 [–0.18–0.14]	NS	0.10 [–0.04–0.24]	NS
Fibrinogen mg·dL <sup>–1</sup>	969	–1.3 [–2.3––0.3]	0.01	–1.4 [–2.5––0.3]	0.01	–0.7 [–2.8–1.5]	NS
PD-FEV1 mL	996	5.1 [0.4–9.7]	0.03	7.2 [1.8–12.5]	0.008	–2.5 [–12.0–7.1]	NS
PD-FEV1 % predicted	996	0.19 [0.04–0.34]	0.02	0.24 [0.07–0.41]	0.006	–0.01 [–0.31–0.30]	NS
PD-FVC mL	996	–0.4 [–7.7–6.9]	NS	0.2 [–8.0–8.6]	NS	–3.8 [–18.9–11.4]	NS
PD-FEV1/FVC %	996	0.2 [0.1–0.3]	0.001	0.2 [0.1–0.4]	<0.001	–0.02 [–0.2–0.2]	NS
ΔFEV1 mL year 1–year 0	996	–1.9 [–4.1–0.03]	NS	–2.0 [–4.2–0.3]	NS	–1.7 [–6.8–3.4]	NS
Exacerbations	996	–0.012 [–0.026–0.002]	NS	–0.017 [–0.034––0.0005]	0.043	0.003 [–0.024–0.030]	NS
PD15 g·L <sup>–1</sup>		0.4 [0.2–0.6]	0.001	0.5 [0.2–0.8]	<0.001	0.08 [–0.04–0.6]	NS
LAA%	948	–0.15 [–0.25––0.04]	0.006	–0.19 [–0.32––0.06]	0.004	0.01 [–0.18–0.20]	NS
ΔLAA% year 1–year 0	887	–0.012 [–0.048–0.024]	NS	–0.021 [–0.065–0.024]	NS	0.017 [–0.047–0.081]	NS
<b>Year 3 (follow-up)</b>							
PD-FEV1 mL	925	5.4 [0.4–10.3]	0.03	7.5 [1.7–13.4]	0.01	–1.3 [–10.8–8.3]	NS
PD-FEV1 % predicted	925	0.21 [0.05–0.37]	0.01	0.28 [0.09–0.47]	0.004	–0.005 [–0.31–0.30]	NS
ΔFEV1 mL year 3–year 1	925	1.0 [–1.2–3.3]	NS	1.6 [–0.9–4.1]	NS	–0.5 [–5.3–4.3]	NS
Exacerbations	995	–0.014 [–0.028–0.00003]	NS	–0.015 [–0.03–0.002]	NS	–0.013 [–0.040–0.015]	NS
LAA%	876	–0.17 [–0.30––0.04]	0.009	–0.19 [–0.3––0.03]	0.02	–0.09 [–0.31–0.12]	NS
ΔLAA% year 3–year 1	876	6.1×10 <sup>–6</sup> [–0.05–0.05]	NS	0.03 [–0.03–0.08]	NS	–0.06 [–0.15–0.02]	NS
Death	996	1.04 <sup>§</sup> [1.01–1.08]	0.02	1.04 <sup>§</sup> [1.01–1.08]	0.02	0.95 <sup>§</sup> [0.74–1.22]	NS

6MWD: 6-min walking distance; mMRC: modified Medical Research Council dyspnoea score; SGRQ: St George's Respiratory Questionnaire score; BODE: body mass index, obstruction, dyspnoea, exercise capacity; PD: post-bronchodilator; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV1: forced expiratory volume in 1 s; LAA%: percentage of low-attenuation areas; CVD: cardiovascular disease; MFAP4: microfibrillar-associated protein 4; IL: interleukin; FVC: forced vital capacity; Δ: change in; PD15: provocative dose causing a 15% fall in FEV1; NS: nonsignificant. <sup>#</sup>: n=966; <sup>¶</sup>: n=629; <sup>+</sup>: n=367; <sup>§</sup>: odds ratio (not B-coefficient).

The median hpMFAP4 was 11.7 U·mL<sup>–1</sup> in first hpMFAP4 quartile (Q1), 19.3 U·mL<sup>–1</sup> in the fourth quartile (Q4) and the range was 2.6–69.2 U·mL<sup>–1</sup>. hpMFAP4 was significantly lowered by current smoking in COPD patients, with 56% current smokers in Q1 and 27% in Q4, respectively (p<0.001). Smoker pack-years did not affect hpMFAP4 significantly and hpMFAP4 levels were stratified by current smoking.

hpMFAP4 associated positively and significantly with 6-min walk distance, post-bronchodilator (PD) forced expired volume in 1 s (FEV1) and PD-FEV1 % predicted, but not with decline in FEV1. Likewise, hpMFAP4 associated with the presence of CVD history (32%) and mortality throughout the 3-year study period (total of 30 participants died) (table 1). These significant positive associations were observed in former smokers but not in current smokers after stratification. In hpMFAP4 Q1, 30% of patients had a history of CVD whereas this fraction was increased to 44% in Q4 (p<0.001). Mortality increased from 1.6% in Q1 to 5.6% in Q4 (p=0.04).

hpMFAP4 associated negatively and significantly with BMI, BODE (BMI, obstruction, dyspnoea, exercise capacity) index, Global Initiative for Chronic Obstructive Lung Disease group 1–4, percentage of low-attenuation areas (LAA%), inflammatory markers and exacerbations, but not with the change in LAA% during the 3-year period. Again, these significant negative associations were only observed in former smokers.

hpMFAP4 associated negatively and significantly with modified Medical Research Council dyspnoea scale and St George's Respiratory Questionnaire (SGRQ), but only in current smokers.

We have shown that elevated hpMFAP4 levels are directly related to the highest lung function measures, the lowest disease grading and emphysema in COPD patients. These relationships were only evident for former smokers and appeared to be disrupted or inverted by current smoking. Although we observed such significant relationships, hpMFAP4 did not appear to be a strong pulmonary marker due to the effect of smoking, and because the variation was not sufficiently strong to predict a fall in lung function or an increase in emphysema development during the 3-year study period. In a previous smaller, yet similar study, we demonstrated that circulating MFAP4 was stable in stable COPD patients but was induced 40–50% 1–6 months after an acute exacerbation. This former study demonstrated significant observations between circulating MFAP4 and various pulmonary outcomes in COPD. However, the previously observed effect sizes were not similar to those currently observed, and circulating MFAP4 was not significantly different between COPD subjects and control subjects [10]. This finding underscores that MFAP4 has no clinical efficacy as marker of lung disease. In line with this notion, circulating MFAP4 did not previously detect any significant changes in idiopathic pulmonary fibrosis, even though a marked significant induction was recognised to occur in the fibrotic lung *in vivo* [14] and in liver parenchyma as well as in blood in liver fibrosis [7].

hpMFAP4 was associated with disease history and death in former smokers, and again the relationship appeared to be disrupted by current smoking. However, a similar association to mortality in peripheral artery disease was previously observed [11] and suggests that the variation of circulating MFAP4, which was also observed in the present study is a consequence of cardiovascular disease.

The strong influence of current smoking on circulating levels appear to hamper the use of MFAP4 in diseases without strong induction of circulating levels, such as in liver fibrosis [7]. Nevertheless, circulating MFAP4 appears to be a relevant biomarker to identify COPD patients at risk of death and cardiovascular comorbidity after smoking cessation. Our observations of MFAP4 association with CVD and death in this cohort resembles previous conclusions obtained when testing desmosine variation [12]. As MFAP4 colocalises with elastin *in vivo* [13] and binds elastin *in vitro* [2], we suggest that the observed significant hpMFAP4 variation predominantly reflects elastin degradation, potentially caused by aberrant inflammation in vascular tissues, contributing to worse cardiovascular outcomes and mortality, in line with the previously observed variation for desmosine.

Our study suggests that hpMFAP4 in patients with COPD is a useful marker of cardiovascular risk and mortality.

**Sofie Lock Johansson<sup>1</sup>, Helle Wulf-Johansson<sup>1</sup>, Anders Schlosser<sup>1</sup>, Ingrid L. Titlestad<sup>2</sup>, Bruce Miller<sup>3</sup>, Ruth Tal-Singer<sup>3</sup>, Uffe Holmskov<sup>1</sup>, Jørgen Vestbo<sup>2,4</sup> and Grith Lykke Sørensen<sup>1</sup>**

<sup>1</sup>Dept of Cancer and Inflammation Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark. <sup>2</sup>Dept of Respiratory Medicine, Odense University Hospital, Odense, Denmark. <sup>3</sup>GlaxoSmithKline R&D, Collegeville, PA, USA. <sup>4</sup>Division of Infection, Immunity and Respiratory Medicine, The University of Manchester, Manchester Academic Health Science Centre, and Manchester NHS Foundation Trust, Manchester, UK.

Correspondence: Grith Lykke Sørensen, Institute of Molecular Medicine, University of Southern Denmark, J.B. Winslows Vej 25.3, 5000 Odense C, Denmark. E-mail: glsorensen@health.sdu.dk

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